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Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS

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Abstract

Soil samples collected from the vicinity of "Manpasand textile industry", located near Ichalkaranji, India were studied for screening and isolation of bacterial strains capable of degradation of textile dyes. A potential strain was selected on the basis of rapid dye degradation and later identified as *Comamonas* sp. UVS. *Comamonas* sp. UVS showed 100% decolorization of Direct Red 5B (DR5B) dye at 40 °C and pH 6.5. The maximum Direct Red 5B concentration decolorized was 1100 mg/l in nutrient broth within 125 h. A numerical simulation with the Michaelis–Menten kinetics model gives an optimal value of 16.01 ± 0.36 mg dye/g cell/h for maximum rate (V_{max}) and 7.97 ± 0.21 mg/l for the Michaelis constant (K_m). The induction in the activities of laccase and LiP was observed during decolorization. These enzymes were inhibited by the addition of sodium azide. The biodegradation was monitored by UV–vis, FTIR spectroscopy and HPLC. The GCMS analysis indicated the presence of 7-benzoylamino-3-diazenyl-4-hydroxy-naphthalene-2-sulfonic acid in degraded product of the dye. The germination of *Triticum aestivum* seeds was inhibited with DR5B treatment but not with the treatment of dye degradation products.

Keywords: Biodegradation; *Comamonas* sp. UVS; Kinetics of dye decolorization

1. Introduction

Large amounts of structurally different dyes are used for textile dyeing and around 10–15% of all dye is directly lost to wastewater [1]. The effluents from textile and dyeing industries have high BOD, COD, color, pH, and also it contains metal ions, hence it is very difficult to treat such effluents [2]. Dye wastewaters are usually treated using physicochemical methods such as flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation ozonization and Fenton's oxidation [3]. Recently, coupled chemical–biological treatment technologies used for the mineralization and/or decolorization of dyes as these technologies are most acceptable for the recalcitrant compounds like dyes [4]. These methods are effective but may generate significant amounts of chemical sludge, whose disposal in secure landfill increases process cost [5].

Microbial decolorization and degradation is an environmental friendly and cost effective alternative to chemical decomposition processes [6]. Wide range of microorganisms is capable of decolorizing a variety of dyes include some bacteria, fungi, yeasts and algae [7]. Various fungal cultures mainly belonging to white rot fungi have been described to oxidize synthetic dyes [8]. However, the majority of white rot fungi described require nitrogen-limiting conditions and long time periods to decolorize dyes, which limits their applications to the laboratory level. In the present study, we have focused our attention on the isolation of dye decolorizing bacterial strains from dye-contaminated soil from an industrial site. We have reported the decolorization ability of the isolated bacteria for several industrial dyes using various conditions. The intermediates formed during the dye degradation have been analyzed.

2. Materials and methods

2.1. Dyes and chemicals

All textile dyes including direct, reactive and dispersive dyes were collected from Manpasand textile industry, located near Ichalkaranji, India. 2,2-Azinobis (3-ethylbenzothiazolin-6-sulfonic acid (ABTS) was obtained from Sigma Chemical

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Company (St. Louis, MO, USA). Tartaric acid was obtained from BDH Chemicals (Mumbai, India). Catechol, *n*-propanol and other fine chemicals were purchased from Sisco Research Laboratory, India. Biochemical test kit was obtained from Himedia (Mumbai, India). All chemicals used were of the highest purity available and of an analytical grade.

2.2. Acclimatization, screening of microorganism and culture conditions

Soil sample collected from dye-contaminated site around Manpasand textile industry was used for screening of dye decolorizing bacterial strains. One gram soil was added to 100 ml nutrient broth containing Direct Red 5B (DR5B, 50 mg/l) and incubated at 30 °C under shaking (120 rpm) as well as static condition. The culture showing decolorization at static condition was acclimatized by transferring 5 ml aliquots from the decolorized flask to the fresh dye-containing medium with various concentration of dye increased from 50 to 500 mg/l. The dye-containing nutrient agar plates were inoculated with 0.1 ml suspension from these flasks. The isolated colonies were transferred to the dye-containing nutrient broth and selected on the basis of rapid decolorization. Out of these isolates one showing faster decolorization under static condition was identified *as Comamonas* sp. UVS and used for further decolorization study. Nutrient medium of composition g/l: peptone 5, beef extract 3, NaCl 5 along with different dyes were used for decolorization study. Decolorization performance of *Comamonas* sp. UVS was also studied in yeast extract broth (composition g/l: yeast extract 10 g), beef extract broth (g/l: beef extract 10) and Luria Bertani broth (composition g/l, casein enzymic hydrolysate 10, yeast extract 5, sodium chloride 10). The pure culture was maintained on dye-containing nutrient agar slants at 4 °C.

2.3. 16S rDNA sequencing

16S rDNA sequencing of isolated bacteria was carried out at GeneOmbio Technologies Pvt. Ltd., India. The nucleotide sequence analysis of the sequence was done at Blast-n site at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST). The alignment of the sequences was done by using CLUSTALW program V1.82 at European bioinformatics site (http://www.ebi.ac.uk/clustalw). The sequence was refined manually after crosschecking with the raw data to remove ambiguities and submitted to the EMBL, the accession number is AM503546. The Phylogenetic tree is constructed using the aligned sequences by the neighbor joining method using Kimura-2-parameter distances in MEGA 2.1 software [9].

2.4. Decolorization experiment

A microbial culture of 0.1 optical density (at 620 nm, 5 ml) was inoculated in 250 ml Erlenmeyer flask containing 100 ml nutrient broth and incubated at 40 °C for 13 h under static condition. After 13 h of incubation, dye was added in individual flasks at a concentration of 50 mg/l. 5 ml sample was withdrawn at different time intervals, centrifuged at 5000 rpm for 15 min. The clear supernatant was used to measure the decolorization at the absorbance maxima of the respective dyes. Uninoculated controls were used to compare color loss during the experiment. All decolorization experiments were performed in three sets, then percentage decolorization was calculated [10] and the average decolorization rate was calculated as follows

$$\text{Average decolorization rate} = \frac{C \times \%D \times 1000}{100 \times t}$$

where C, initial concentration of dye (mg/l); $\%D$, dye decolorization (%) after time t.

As DR5B required less time for complete decolorization, it was used for further study. Nutrient broth grown *Comamonas* sp. UVS (13 h) was used to study the effect of static and shaking (120 rpm) condition, various temperatures (30–60 °C at pH 6.5) and pH (2.0–14.0 at 40 °C) on decolorization of DR5B (50 mg/l). Reduction in chemical oxygen demand (COD) [11] was also studied. Decolorization at increasing concentrations of DR5B (50–1100 mg/l) was studied at 40 °C and pH 6.5. Decolorization efficiency of the microorganism was reported by repeated use of same culture upto 13 cycles, at static condition.

2.5. Preparation of cell free extract

Comamonas sp. UVS was grown in nutrient broth at 40 °C for 13 h (mid of exponential phase). Cells were collected by centrifugation at 7000 rpm for 20 min and suspended (75 mg/ml) in 50 mM potassium phosphate buffer (7.4 pH) for sonication (sonics-vibracell ultrasonic processor), keeping sonifier output at 60 A, giving 8 strokes, each of 30 s with 2 min interval. The temperature was maintained below 4 °C. This extract was used as source of enzyme.

2.6. Enzyme analysis

Activities of lignin peroxidase (LiP), laccase and tyrosinase were assayed spectrophotometrically in cell free extract and culture supernatant. LiP activity was determined by monitoring the formation of propanaldehyde at 300 nm as reported earlier [12]. Laccase activity was determined by measuring oxidation of ABTS at 420 nm [13]. Tyrosinase activity was determined in a reaction mixture of 2 ml; containing 0.01% catechol in 0.1 M phosphate buffer (pH 7.4) by measuring liberated catechol quinone at 410 nm [14]. All enzyme assays were carried out at room temperature; reference blanks contained all components except the enzyme. One unit of enzyme activity was defined as a change in absorbance unit per ml of enzyme.

2.7. Decolorization and biodegradation studies

Decolorization was monitored by UV–vis spectroscopic analysis (Hitachi U-2800), whereas biodegradation was monitored by HPLC and FTIR. Identification of metabolites was carried out by GCMS. After complete decolorization of DR5B, the decolorized medium was centrifuged at 5000 rpm for 20 min and the supernatant obtained was used to extract metabolites with equal volume of ethyl acetate. The extracts were dried over anhy-

Table 1
Morphological characters of *Comamonas* sp. UVS

Size	Shape	Color	Margin	Elevation	Opacity	Consistancy	Gram nature	Motility
2 mm	Circular	White	Entire	Flat	Opaque	Moist	Gram negative	Motile

drous Na_2SO_4 and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of HPLC grade methanol and used for HPLC, FTIR and GCMS analysis. High performance liquid chromatography (HPLC) analysis was carried out (waters model no. 2690) on C_{18} column (symmetry, 4.6 mm × 250 mm) by using gradient of methanol and acetonitrile (75:25) with flow rate of 1 ml/min for 10 min and UV detector at 254 nm.

The Fourier Transform Infrared Spectroscopy (FTIR) analysis of extracted metabolites was done on PerkinElmer, Spectrum one instrument and compared with control dye in the mid IR region of 400–4000 cm^{-1} with 16 scan speed. The samples were mixed with spectroscopically pure KBr in the ratio 5:95, pellets were fixed in sample holder, and the analysis was carried out. GCMS analysis of metabolites was carried out using a Shimadzu 2010 MS Engine, equipped with integrated gas chromatograph with a HP1 column (60 m long, 0.25 mm i.d., nonpolar). Helium was used as carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 280 °C with oven conditions as: 80 °C kept constant for 2 min – increased upto 200 °C with 10 °C/min – raised upto 280 °C with 20 °C/min rate. The compounds were identified on the basis of mass spectra and using the NIST library.

2.8. Effect of inhibitor on decolorization and enzyme activity of cells

Effect of sodium azide (0–0.5 mM), on DR5B decolorization as well as on LiP and laccase activity was studied. Sodium azide was added in the nutrient broth during the growth of *Comamonas* sp. UVS.

2.9. Phytotoxicity study

The ethyl acetate extracted products of DR5B degradation were dried and dissolved in 10 ml sterile distilled water to make a final concentration of 5000 ppm for phytotoxicity studies. The phytotoxicity study was carried out (at room temperature) in relation to *Triticum aestivum* (10 seeds) by watering separately 10 ml sample of control DR5B and its degradation products (5000 ppm) per day. Control set was carried out using distilled water at the same time. Germination (%) and length of plumule and radical was recorded after 7 days.

2.10. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons. Readings were considered significant when P was ≤ 0.05.

3. Results and discussion

3.1. Isolation and screening of dye decolorizing bacteria

A potential organism was selected on the basis of time required for decolorization of dye DR5B. The organism showed best growth in nutrient broth at 40 °C and pH 6.5. An identification of the bacterial isolate was done on the basis of biochemical tests and 16S rDNA analysis. The bacterium was Gram negative, rod shaped motile organism. Other morphological characters are presented in (Table 1). Various biochemical tests were performed for the identification and characterization of bacteria by using Himedia kit (Table 2). We have checked the ability of this isolate to utilize various sugars (Table 3). The sequence of the 16S rRNA gene of the strain *Comamonas* sp. UVS is available under the EMBL with accession number AM503546. After the initial analysis at NCBI, the relevant sequences were downloaded and phylogenetic analysis was done (Fig. 1). In the phylogenetic tree, the isolate clustered within the family *Comamonadaceae* of *proteobacter*. Within this family, it showed a lineage distinct from species *Comamonas testosteroni*. The same branch also contained *Comamonas* sp. AV1A. The separation of the isolate UVS from this genus was supported by bootstrap value of 99%. Phylogenetic analysis using other methods of tree building also supported this grouping with high bootstrap values. The similarity of 16S rDNA sequence supported the assignment of the strain UVS to the genus *Comamonas* and designated as *Comamonas* sp. strain UVS. *Comamonas* sp. was previously known for degradation of naphthalene and aniline [15,16]**.** Our studies revealed that *Comamonas* sp. UVS could not use dye as a sole carbon and energy source. The decolorization of dye occurred only when nutrient broth was supplemented as growth medium. Along with nutrient broth we have tested the effect of various media on the decolorization performance of *Comamonas* sp.

Table 2
Biochemical tests for the identification and characterization of *Comamonas* sp. UVS

Sr. No.	Biochemical test	Result
1	Citrate utilization	Positive
2	Lysine decarboxylation	Positive
3	Ornithine decarboxylation	Positive
4	Urease	Negative
5	Phenylalanine deamination	Negative
6	Nitrate reduction	Positive
7	H_2S production	Negative
8	Glucose	Positive
9	Adonitol	Negative
10	Lactose	Positive
11	Arabinose	Positive
12	Sorbitol	Negative

Table 3
Tests for utilization of various sugars by *Comamonas* sp. UVS

Sugar	Result	Sugar	Result	Sugar	Result
Xylose	Positive	Inulin	Negative	Rhanose	Negative
Maltose	Positive	Sodium gluconate	Negative	Cellobiose	Positive
Fructose	Negative	Glycerol	Negative	Melezitose	Negative
Dextrose	Positive	Salicin	Negative	α-Methyl-D-mannoside	Negative
Galactose	Positive	Glucosamine	Negative	Xylitol	Negative
Raffinose	Negative	Dulictol	Negative	ONPG	Negative
Trehalose	Positive	Inositol	Negative	Malonate	Positive
Melibiose	Positive	Mannitol	Negative	Esculin	Negative
Sucrose	Negative	α-Methyl-D-glucoside	Negative	Sorbose	Negative
Mannose	Positive	Ribose	Positive		

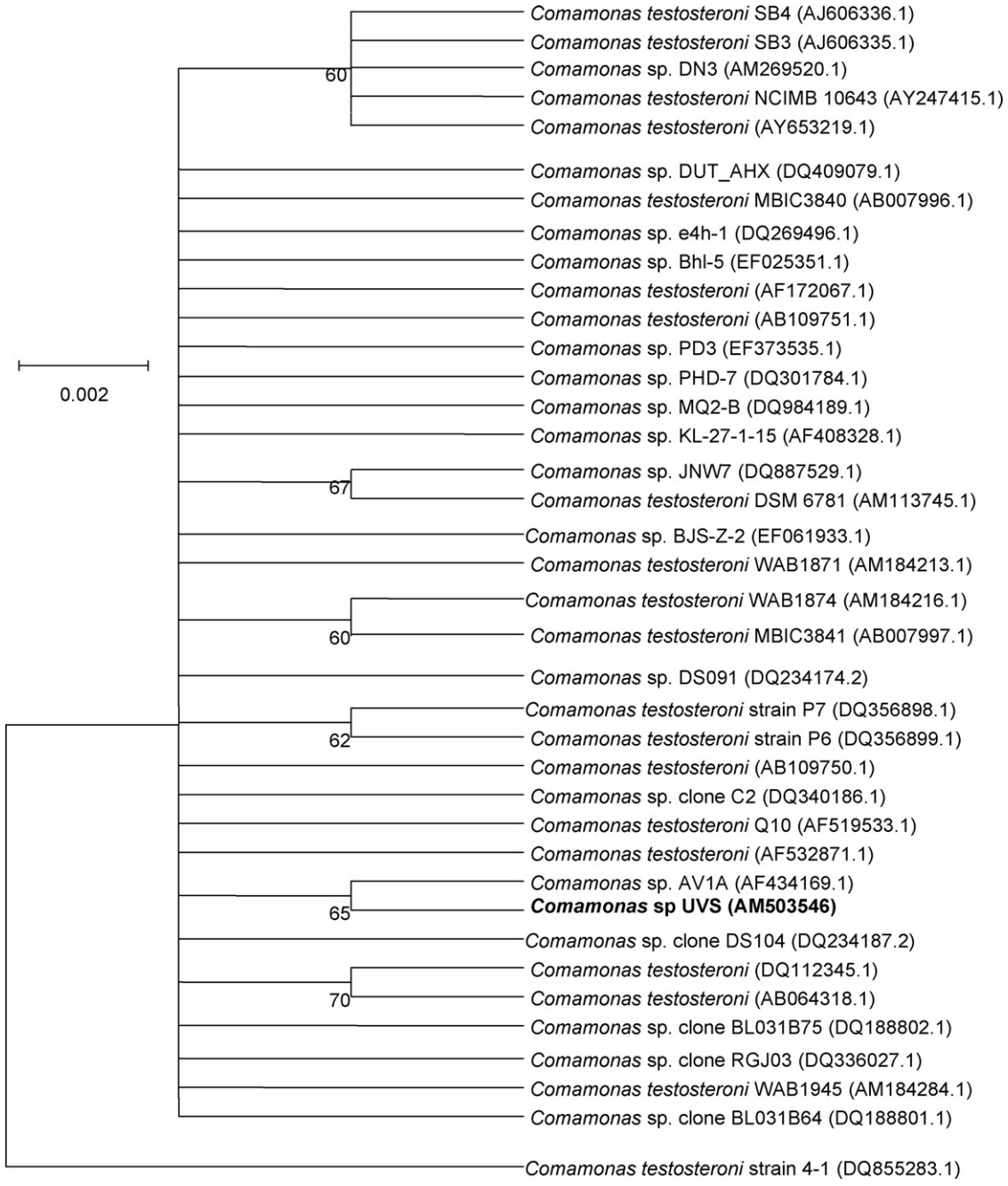


Fig. 1. Phylogenetic tree of the *Comamonas* sp. UVS and related organisms were aligned based on 16S rDNA sequences (neighbor-joining tree). Scale bar: number of nucleotide changes per sequence position. The number at nodes shows the bootstrap values obtained with 1000 resampling analyses.

Table 4
Decolorization performance of *Comamonas* sp. UVS in various media

Media	Time of decolorization (h)
Nutrient broth	13
Lauria Bertani broth	8
Yeast extract broth	6
Beef extract broth	30

UVS. In yeast extract broth DR5B was decolorized within 6 h (Table 4).

3.2. Effect of temperature and pH on dye decolorization

The dye decolorization activity of *Comamonas* sp. UVS was found to increase with increase in incubation temperature from 25 to 40 °C. Time required for decolorization of DR5B was decreased with the increasing temperature. Further increase in temperature above 40 °C resulted in marginal decrease in decolorization activity (Fig. 2). Decrease in decolorization activity at higher temperature can be attributed to the loss of cell viability [17]. *Comamonas* sp. UVS has the ability to decolorize the dye between the temperature range 25 and 50 °C. The optimum temperature found was 40 °C. Bacterial cultures generally exhibit maximum decolorization at pH values near neutrality. *K. Pneumoniae* RS-13 completely degraded methyl red in pH range from 6 to 8 [18]. Since textile effluents usually do not have a neutral pH, we were interested in testing the ability of *Comamonas* sp. UVS to decolorize DR5B at different pH values. *Comamonas* sp. UVS has the ability to decolorize the dye within the pH range of 6–12 (Fig. 2). The growth of *Comamonas* sp. UVS did not occur below pH 6 and above pH 12. Fig. 2 shows that, pH 6.5 was best for decolorizing the dye, but pH 12 appeared to be tolerated well and dye could be decolorized at that pH. However to achieve best decolorization, it is suggested that the pH of the textile effluent be neutralized to around 6.5. Our

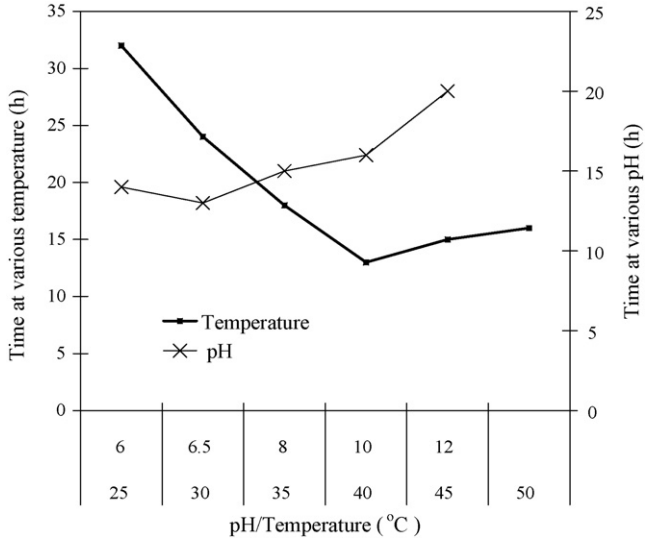


Fig. 2. Decolorization performance of *Comamonas* sp. UVS at various temperature and pH.

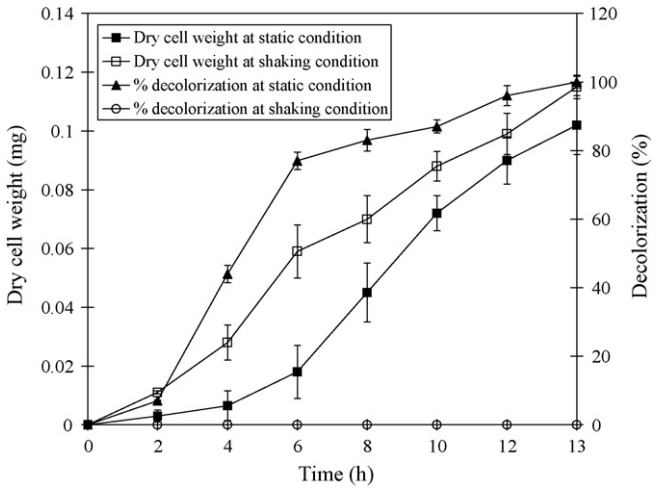


Fig. 3. Effect of static and shaking condition on decolorization of DR5B.

results of temperature and pH are comparable with Mali et al. [19] who found the maximum potential of *Pseudomonas* sp. to decolorize the dye at 37 °C and within a pH range of 6–9.

3.3. Decolorization performance of Comamonas sp. UVS under static and shaking conditions and removal of COD

Comamonas sp. UVS exhibited dye-decolorizing activity only when incubated under static conditions, whereas agitated cultures grew well but showed no decolorization (Fig. 3). At shaking condition there could be competition of oxygen and the azo compounds for the reduced electron carriers under aerobic condition [20]. At static condition 100% decolorization of DR5B was observed within 13 h. The average rate of decolorization was increased up to 6 h and further decreased at 13 h. Removal of COD was 94% at static condition (Fig. 4). Isik and Sponza [21] reported 57% removal of COD at anaerobic condition while 87% and 78% at aerobic and

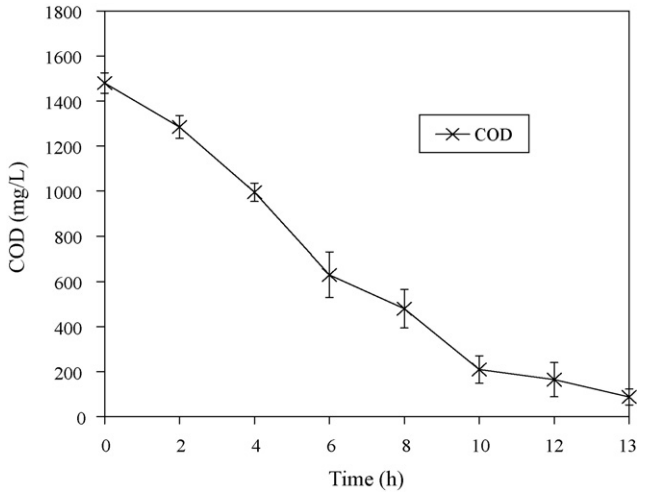


Fig. 4. Removal of COD during decolorization of DR5B.

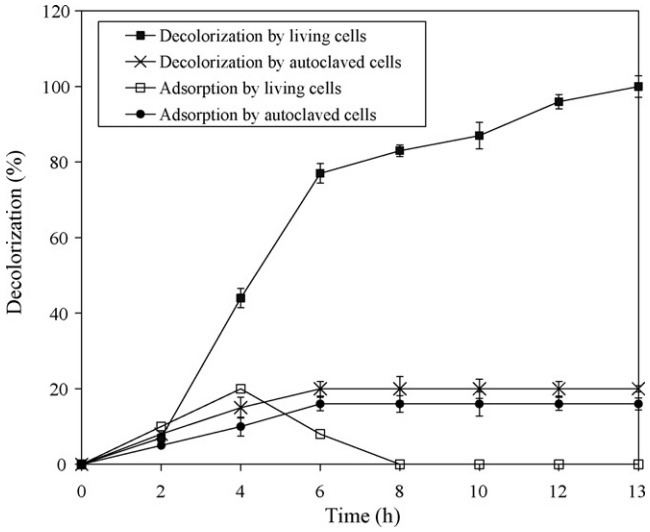


Fig. 5. Decolorization profile of DR5B by living and autoclaved cells of *Comamonas* sp. UVS.

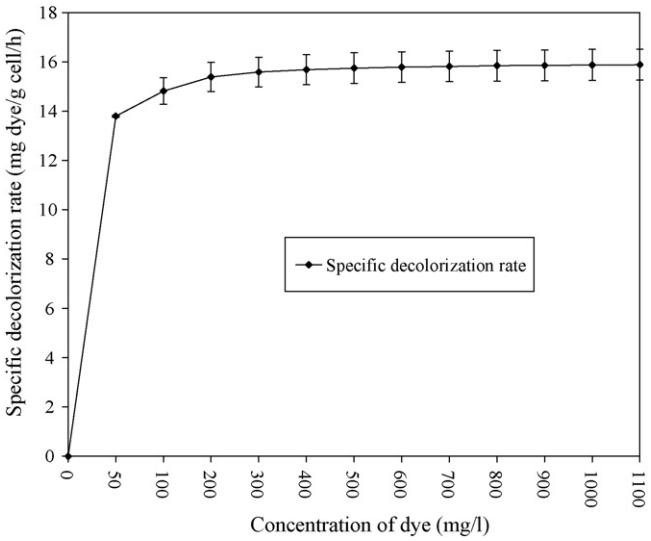


Fig. 6. Effect of dye concentration on specific decolorization rate.

microaerophilic conditions, respectively. The significant reduction in COD showed mineralization of DR5B by *Comamonas* sp. UVS.

3.4. Decolorization by living and autoclaved cells of *Comamonas* sp. UVS

The results in Fig. 5 show initial decolorization of DR5B by autoclaved cells of *Comamonas* sp. UVS (20%) during the first 6 h, but no further significant variation in decolorization was observed during the next 13 h. Extraction with methanol recovered the major part of the decolorized dye, indicating that DR5B decolorization by autoclaved cells of *Comamonas* sp. UVS was mainly due to adsorption. In contrast, living cells of *Comamonas* sp. UVS were able to decolorize DR5B completely within 13 h of incubation. It was shown that the initial dye decolorization by living cells was partly due to adsorption but that the eventual fraction of methanol-extractable dye was negligible. These results clearly indicate that the overall decolorization of DR5B by *Comamonas* sp. UVS was due to biological mechanisms and not due to adsorption. Similar observations were reported earlier by Khehra et al. [22].

3.5. Effect of dye concentration on decolorization and its kinetics

The decolorization activity of *Comamonas* sp. UVS was studied using DR5B at various initial dye concentrations. *Comamonas* sp. UVS can decolorize up to 1100 mg/l of dye, however higher concentration seemed to be toxic for cell growth in 100 ml batch culture. The dye decolorization was strongly inhibited at 1200 mg/l dye in the medium. Similar results were also obtained earlier for other dyes [23,24]. By considering *Comamonas* sp. UVS as a whole cell enzyme, conventional Michaelis–Menten kinetics ($V = V_{max}\ S/(K_m + S)$) can be used to describe the dye decolorization kinetics. A numerical simulation with the model gave an optimal value of 16.01 (±0.36) mg dye/g cell/h for maximum rate (V_{max}) and 7.97 (±0.21) mg/l for the Michaelis constant (K_m). The study of dye decolorization kinetics showed that the specific decolorization rate increases with increase in initial dye concentration and then it slowed down (Fig. 6). Chang and Lin [25] showed that the decolorization rate increased linearly with increase in initial dye concentration up to certain extent and then it leveled off. Reduction in the decolorization rates may result due to toxicity of the dye to bacteria and/or inadequate biomass concentration for the uptake of higher concentrations of dye [26]. Our observations are comparable to the work carried out on decolorization of same dye DR5B with a bacterial consortium NBNJ6 [27].

3.6. Decolorization of repeated addition of dye aliquots and various dyes

In the commercial point of view the repeated use of microorganisms is important [28]. This study was carried out to test the ability of *Comamonas* sp. UVS to decolorize repeated addition of DR5B aliquots at static condition. There was 100% decolorization for first dye aliquot addition within 13 h. When second aliquot of dye was added, this was decolorized (86.84%) within next 5 h (Fig. 7). This repeated batch operations could decolorize the dye at a much faster rate in subsequent addition maintaining high decolorization activity (>72%). The eventual cessation of decolorization is likely to be due to nutrient depletion. The results indicate that *Comamonas* sp. UVS holds excellent stability and persistence in repetitive decolorization operations. This would increase the applicability of using the strain in practical wastewater decolorization. Repeated use of microbial cells was also reported by other workers [29,30].

The capacity of microorganism can be tested by examining its potential to degrade various dyes [31]. *Comamonas* sp. UVS can degrade 13 various textile dyes (50 mg/l each) in nutrient broth at static condition. The time required for dye decolorization and percentage decolorization were shown in Table 5.

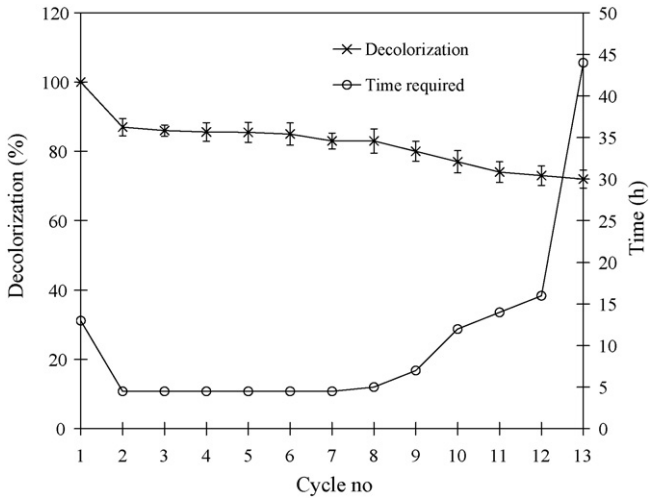


Fig. 7. Repeated use of *Comamonas* sp. UVS in nutrient broth for decolorization of DR5B.

3.7. Enzyme activities while decolorization in batch culture

It can be presumed that the major mechanism of decolorization in microorganisms is mostly because of the biotransformation enzymes like lignin peroxidase, laccase to mineralize the synthetic dyes [32]. The role of lignin peroxidase, laccase in the decolorization of dyes may be different for each microorganism [33]. Khandelbauer et al. [34] reported the role of fungal peroxidase and laccases for the oxidation of sulfonated azo dyes. Azo dye degrading bacteria were also able to produce peroxidase [35]. The time course of LiP, laccase as well as tyrosinase was studied for *Comamonas* sp. UVS during decolorization of DR5B. Cell lysate of *Comamonas* sp. UVS has shown presence of LiP and laccase activity, during decolorization. The induction in the activity of LiP and laccase were observed after 3 and 6 h of addition of the dye, respectively (Fig. 8). The activities of these enzymes were decreased within 9–13 h (when complete decolorization occurs). The presence of very low concentration of dye within 9–13 h could be the reason for decrease in the enzyme activities. Kalme et al. [36] also reported similar observation of induction in extracellular LiP, intracellular laccase and tyrosinase activities. The activity of tyrosinase was not significant. No lignin peroxidase, laccase and tyrosinase activity was observed in culture supernatants.

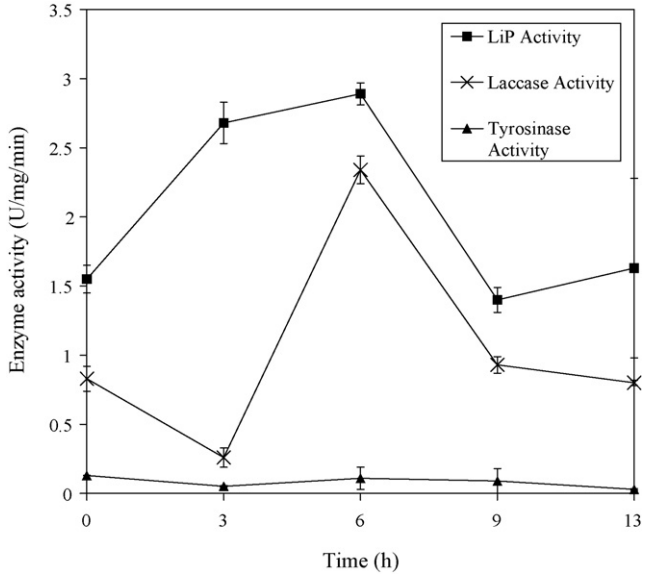


Fig. 8. Time course of lignin peroxidase, laccase and tyrosinase activities during decolorization of DR5B in nutrient broth.

Table 5
Percent decolorization of different textile dyes by *Comamonas* sp. UVS

Sr. No.	Name of dyes	λ_{max} (nm)	Time (h)	Decolorization (%)
1	Direct Brown MR	400	48	93
2	Direct Orange T4	420	48	87
3	Direct yellow 5GL	501	48	93
4	Disperse Golden yellow	410	48	95
5	Brilliant Blue GRL	485	48	73
6	Reactive Blue HERD	615	48	95
7	Golden Yellow HER	430	48	90
8	Blue 2RNL	560	96	78
9	Navy Blue 2GL	535	96	80
10	Navy Blue HE2R	570	96	73
11	DK Red 2B	535	96	75
12	Navy Blue RX	576	168	70
13	Red HE8B	560	192	65

3.8. Effect of inhibitor on production of enzymes and decolorization

The effect of inhibitor sodium azide (0.5 mM, which was not inhibitory to growth of *Comamonas* sp. UVS) on the specific activity of LiP and laccase of the cells was tested by using *n*-propanol and ABTS as the substrates. The aim of this study was to prove the involvement of ligninolytic enzymes in decolorization of DR5B dye. Sodium azide (0.5 mM in the medium) showed (89%) decrease in LiP and 56% in laccase activity when recorded at 13 h. It also showed decrease in % decolorization of DR5B (Fig. 9). Similar inhibition of enzyme by sodium azide was also reported by Novotny et al. [37].

3.9. Phytotoxicity study

Despite the fact, untreated dyeing effluents may cause the serious environmental and health hazards. They are being disposed off in water bodies and this water can be used for the agriculture purpose. Use of untreated and treated dyeing effluents in agriculture has direct impact on fertility of soil. Thus, it was of concern to assess the phytotoxicity of the dye before and after degradation. The relative sensitivity towards the dye DR5B and its degradation products in relation to *T. aestivum* was studied. The mean of plumule length and radical length of *T. aestivum* was 10.4 ± 0.13 and 10.4 ± 0.06 cm, respectively of 10 seeds in distilled water as a control with 100% germination. The seed germination was completely inhibited, when seeds were treated with 5000 ppm concentration of DR5B, where as the plumule length and radical length was found to be 10 ± 0.43 and 10.3 ± 0.17 cm, respectively with 100% germination when treated with 5000 ppm degradation

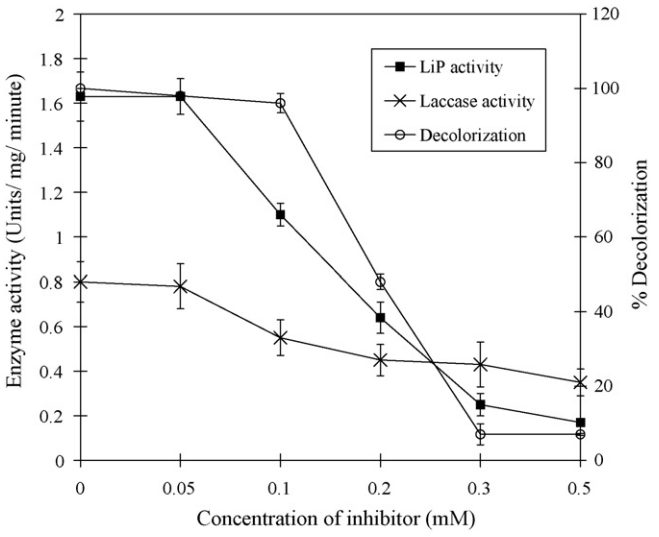


Fig. 9. Inhibition of oxidative enzymes and decreased decolorization due to increasing concentration of sodium azide. The activity of enzyme and % decolorization of dye observed at 13 h.

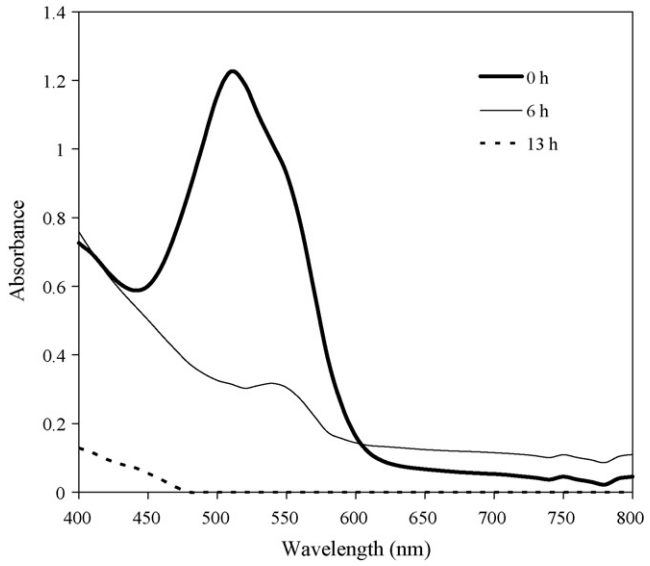


Fig. 10. UV–vis spectra at different time interval during decolorization of DR5B.

product. This indicates the less toxic nature of the degradation product to the plants. Parshetti et al. [38] also showed germination of *T. aestivum* was less with malachite green treatment as compared to its degradation product and distilled water.

3.10. Degradation product analysis

UV–vis analysis (400–800) of supernatants of different time intervals showed decolorization and decrease in dye concentration from batch culture. Peak observed at 512 nm (0 h) was decreased without any shift in λ_{max} up to complete decolorization of medium (13 h) (Fig. 10). According to Asad et al. [39] decolorization of dyes by bacteria could be due to adsorption by microbial cells, or to biodegradation. In the case of adsorption, the UV–vis absorption peaks decrease approximately in proportion to each other, whereas in biodegradation, either the major visible light absorbance peak disappears completely, or a new peak appears. The observation of cell mass showed that *Comamonas* sp. UVS retained their natural color after decolorization of DR5B.

Comparison of FTIR spectrum of control dye with extracted metabolites after complete decolorization clearly indicated the biodegradation of the dye by *Comamonas* sp. UVS (Fig. 11). Peaks in the control dye spectrum represented the deformation of C–H at 715 cm^{-1}, stretching of P–O bond at

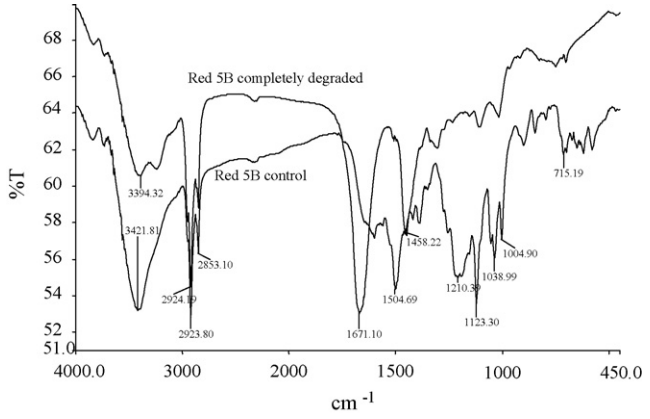


Fig. 11. FTIR analysis of control DR5B and degradation products.

1004 and 1038 cm^{-1}. The stretching vibrations at 1123 cm^{-1} showed C–OH stretching. We observed S=O stretching at 1210 cm^{-1} and N–H deformation at 1504 cm^{-1}. At 2924 cm^{-1} showed C–H asymmetric stretching and the free N–H group showed amide band at 3421 cm^{-1}. The FTIR spectrum of 13 h extracted metabolites showed significant change in positions of peaks when compared to control dye spectrum. A new peak at 1458 cm^{-1} represented C–H, asymmetric deformation of alkane, where as peak at 1671 cm^{-1} was observed for C=N stretching. Peak at 2853 and 2923 cm^{-1} represented C–H deformation. A peak at 3394 cm^{-1} showed N–H stretch-

Table 6
GC–mass spectral data of biodegraded products of DR5B

Sr. No.	Rt (min)	MW	Relative abundance in mass spectrum: *m/z* (% relative intensity)	Proposed compound
1	17.333	(*m/z* + 1) 381	70 (80), 86 (30), 154 (100	7-Benzoylamino-3-diazenyl-4-hydroxy-naphthalene-2-sulfonic acid

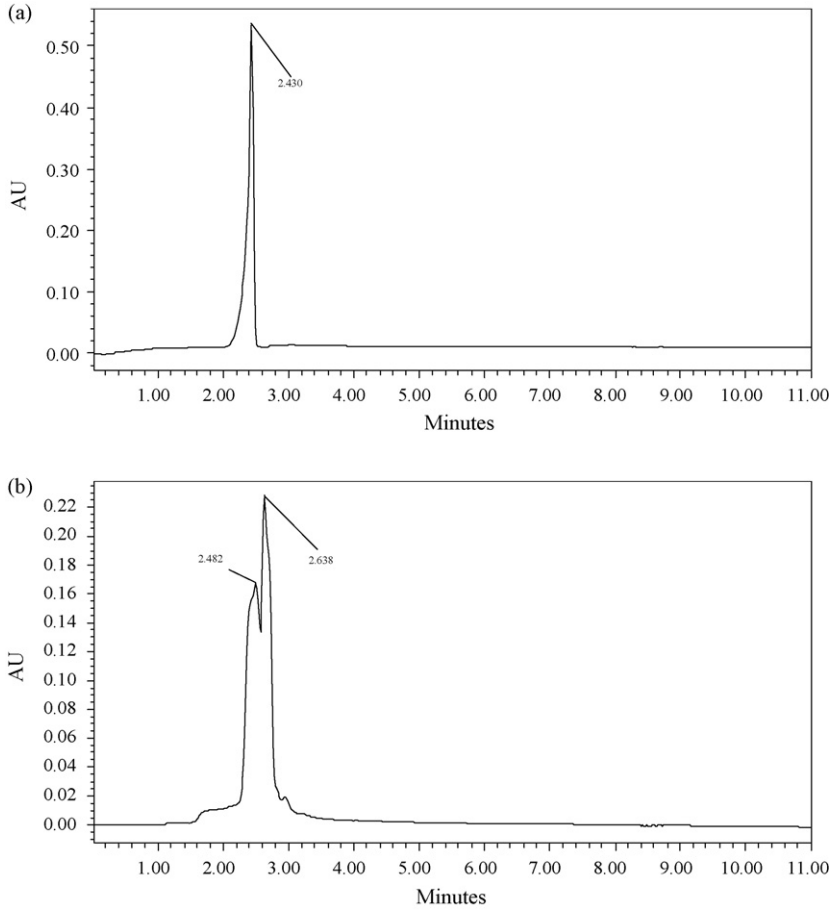
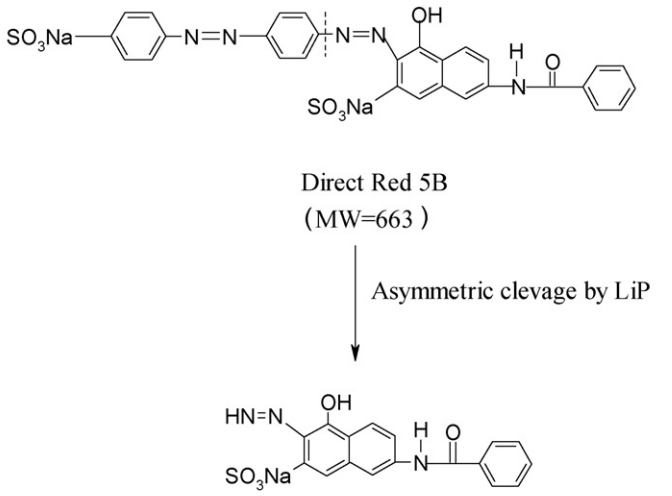


Fig. 12. HPLC analysis of control DR5B (a) and degradation products (b).



7-benzoylamino-3 diazenyl-4 hydroxy-naphthalene-2-sulfonic acid

[MW (mz+1)=381]

Fig. 13. Biodegradation pathway of DR5B.

ing. HPLC analysis of extracted sample showed original dye at retention time 2.430 min (Fig. 12a) and after complete decolorization peaks for metabolites were at retention time 2.482 and 2.638 min (Fig. 12b). GCMS analysis was carried out to investigate the metabolites formed during the biodegradation process (Table 6). Azo dyes can be cleaved symmetrically or asymmetrically, with an active site available for an enzyme to excite the molecule [40]. A pathway has been proposed for degradation of DR5B by *Comamonas* sp. UVS in static anoxic condition (Fig. 13). The asymmetric cleavage by LiP between the nitrogen of the azo group and the carbon of the aromatic ring resulted in the formation of 7-benzoylamino-3-diazenyl-4-hydroxy-naphthalene-2-sulfonic acid. Recently the LiP mediated asymmetric cleavage of dye was reported by the study of Dawkar et al. [41]. The structure of detected compound was assigned from the *m*/*z* value obtained. GC showed 8 peaks, however we can identify only one product.

4. Conclusion

An isolated microorganism *Comamonas* sp. UVS has the ability to decolorize and degrade DR5B into nontoxic metabolites. Induction in the activities of LiP and laccase was observed during decolorization of DR5B. Also significant inhibition in the activities of LiP and laccase in presence of sodium azide along with significant decrease in dye decolorization indicates major role of these enzymes in the dye decolorization process. Ability of this organism to decolorize various textile dyes indicates its commercial applicability.

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